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Development of the Native Legume *Glycyrrhiza Lepidota* as a Potential Soil Reclamation and Forage Crop

Conducted by Dr. A. Walther

Funded by the Agriculture Development Fund

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DEVELOPMENT OF THE NATIVE LEGUME *GLYCYRRHIZA LEPIDOTA* AS A POTENTIAL SOIL RECLAMATION AND FORAGE CROP.

Walther, A.
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To contract clause 21 a, Executive Summary:

1. The intent was to ascertain salt tolerance of wild licorice. Seed from various populations in southern Saskatchewan were germinated in low to high sodium chloride or sodium sulfate solutions. Concentrations equivalent to severe soil salinity reduced seedling shoot growth but not the development of the taproot and adventitious buds from which new shoots arise. Tests in sulfate-rich soil extract and in saline soils up to 14 mS cm^{-1} confirmed that sulfate is more stressful than chloride although inherent variability amongst seed sources was observed. Wild licorice is recommended for reclamation purposes on moderate to mildly severe salinized sites.

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Executive Summary Continued:

2. The study was suggested by Mr. Don Gayton, Forage and Rangeland specialist, Agriculture Saskatchewan. Wild licorice (Glycyrrhiza lepidota) is a deep-rooted perennial herbaceous legume widely distributed in Saskatchewan, occasionally occurring in stands of salt-tolerant native grasses. The green leafy tops of this plant are consumed by wild herbivores and domestic livestock while the protein-rich seeds are winter food for wildlife. Since the species exhibits some salt tolerance it warranted further investigation.

Mature field-ripened seeds were collected from fourteen different areas in southern Saskatchewan, five of which represented two crop years. Wild licorice seed has a hard water-impermeable seed coat but imbibes and germinates readily when this is abrased. In order to ascertain salt tolerance, seeds were germinated in sodium chloride or sodium sulfate solutions of increasing strength. Germinability was high in all but only 50% or less viable seedlings were obtained in salt concentrations equivalent to severe soil salinity. Growth response to continuous salinity stress under a controlled light and temperature regime was monitored. At moderate salinity levels seedling leaf chlorophyll, stem elongation and shoot mass were depressed becoming more pronounced at severe salinity stress. But the enlargement of the primary seedling root, due to reserve deposition, and formation of adventitious buds from which new shoots arise in this perennial plant species, were only slightly affected by severe salt stress. Discrepancies in the growth response to salinity stress amongst the seed sources tested are ascribed to site conditions during seed formation and inherent variability within populations of wild licorice.

Selected seed sources were also tested in a sulfate-rich soil extract of 17 mS cm^{-1} and in saline soil mixtures up to 14 mS cm^{-1} (the latter under controlled indoor conditions and out-of-doors), and the depressing effect of high salinity

Executive Summary #2 Continued:

levels was similar. The combined data indicates that the sulfate ion is more stressful than the chloride ion. None of the tested seed sources was found to have a low or very high salt tolerance but the results indicate that wild licorice is tolerant of moderate to mildly severe salinity (according to soil salinity categorization by agriculturists). It is concluded that this native plant species can be used for reclamation purposes of salinized patches in otherwise nonsaline soils.

Once established from seed, the combined characteristics of the storage taproot and adventitious buds promote spread by vegetative means. Nitrogen-fixing root nodules have been indicated for this leguminous plant which suggests that local soil nutrient improvement could result from newly established populations. This warrants further research as well as other aspects of this plant that is closely related to the Eurasian species from which commercial licorice is obtained (pertaining to this, even at high sodium chloride concentrations the taproot tasted 'sweet'). Minor economic benefits might accrue in addition to the grazing value of wild licorice on pastures with secondary salinization.

ABSTRACT

Seed of Glycyrrhiza lepidota from widely separated areas in southern Saskatchewan germinated readily in graded sodium chloride or sodium sulfate solutions although the formation of viable seedlings declined significantly in high salt concentrations. Seedling growth under controlled conditions in vermiculite irrigated with 1/2-strength NaCl - or Na_2SO_4-enriched nutrient solutions showed retarded shoot growth. The deposition of reserves in the primary seedling taproot and the formation of adventitious buds, from which secondary shoots arise, was only slightly affected by moderate to lightly severe salinities. Seedling chlorophyll synthesis in the cotyledons and first-formed foliage leaves was progressively depressed at salt concentrations designated as severe, accounting for the retardation of seedling growth.

Germination in saline soils with a high sulfate content was not impaired in mixtures having conductivities up to 7 mS cm^{-1} but declined significantly in soil approaching 14 mS cm^{-1}. Under controlled conditions and in out-of-door tests comparable growth and development were observed.

Response to the artificially imposed or natural soil salinity stress was not uniform amongst the various seed sources reflecting site conditions during seed formation and genetic variability within populations. The germination behaviour and growth performance of seedlings monitored in this study indicate that wild licorice can colonize by establishment from seed, and subsequent spread by vegetative means, sites with moderate to lightly severe soil salinity.

BACKGROUND AND OBJECTIVES

An arbitary criterion used to distinguish nonsaline from saline soils is based on the quantities of soluble salts that interfere with the growth of most crop and forage plants. Such amounts are not fixed but depend on the texture, water capacity and salt composition of the soil, and on the plant species. Cultivated and wild plants differ greatly in their growth response to salt stress. Most wild plants are nonhalophytes e.g., having either relatively low salt tolerance or their growth is severely inhibited even at low salinity levels.

One native prairie plant species that exhibits some inherent salt tolerance, as evidenced by its occasional occurrence on mildly saline sites, is wild licorice (Glycyrrhiza lepidota). It is a deeprooted perennial herbaceous legume that spreads to new sites by seed, and once established, proliferates locally by vegetative means from the underground stems. It forms erect, often profusely branched shoots in the spring. The terminal inflorescences on the main branches contribute to abundant seed production. It is a versatile plant species growing in the open on moist to dry sites, and on various soil types. The green leafy tops are readily consumed by wild herbivores and domestic livestock while the pods with the proteinaceous seeds constitute winter food for wildlife.

The intent of the present investigation was to determine degrees of salt tolereance amongst different seed sources by their germinability and seedling growth response under induced sodium chloride or sodium sulfate stress, and to carry out preliminary germination trials in soils having different salinity levels. The findings to be considered in assessing the feasibility of using this native plant species for reclamation purposes of salinized sites in view of the increasing areas of prairie farm and grazing lands going beyond the salinity tolerance range of traditional crop and forage varieties.

EXPERIMENTAL PROCEDURES

The experimental approach involved standard techniques associated with preparations of solutions, weighings and other routine laboratory procedures.

Chlorophyll was extracted from freshly harvested cotyledons (without their short petioles) and leaves (with their relatively long, green petioles) with 80% acetone, measured spectrophotometrically and calculated as mg Chl a, Chl b, and total chlorophyll per gram leaf tissue (Arnon, 1949). In addition, total chlorophyll was recalcultated on the basis of cotyledon or leaf mass and expressed as total chlorophyll per organ.

For dry weight determinations, individual seedling shoots were cut at the cotyledonary node, and the root systems excavated. The enlarged primary seedling root was stripped of remaining (incomplete) feeding roots, and the root mass here referred to is the primary seedling root, a taproot. All plant matter was dried at 80 ^{o}C to constant weight.

RESULTS AND DISCUSSION

are given in sections entitled:

Seed source

Germination

Growth response of wild licorice to salinity stress

SEED SOURCE

Mature, field-ripened fruits of wild licorice were collected from various populations in southern Saskatchewan. An attempt was made to obtain seed from representative areas with reference to soil salinity risk per Agriculture Saskatchewan map 1984.

The prickly indehiscent pods were opened by hand, and upon visual inspection intact seed separated. Since overwintering in the field does not affect the viability of wild licorice seed, some collections were made in the spring of 1987 (1986 growing season), fall of 1987 and 1988. The yield of intact seed varied both with site and year. Wild licorice seed is subject to predispersal seed predation, and in southern Saskatchewan primarily by a host-specific bruchid, Acanthoscelides aureolus (Walther, 1979). The yield of intact seed was least in collections from sites near the Old Wives Lake and greatest from a small population in a domestic garden (designated 'Bell') in the City of Regina. The latter served as a non-stressed control seed source. The source of seed was identified by the nearest civic center or geographical feature, and by year.

GERMINATION

The seed of wild licorice is hard and small, ranging from 1 - 2 mm in length, and average air dry weights commonly range from 6.5 ± .31 to 6.9 ± .25 mg (Walther, 1979). The hard, water-impermeable seed coat had to be scarified manually to facilitate uniform imbibition. (Seeds were surface-sterilized with ethanol prior to scarifying).

Germination tests in aqueous NaCl or Na_2SO_4 and aqueous sulfate-rich soil extract were carried out at a 14 h photoperiod / 25 °C constant in a low-light intensity incubator. Germination tests in soil were carried out in a growth chamber under a 14 h photoperiod at 25 °C/10h at 15 °C, and out-of-doors in midsummer of 1989 (in the City of Regina). Seeds were placed on double-layered Whatman No. 1 filter paper wetted with a given solution (double-distilled water serving as control) in glass petri dishes; in soil seeds were placed to about 1 cm depth.

Successful germination on filter paper was defined by the visible protrusion of the radicle, to at least 2 mm, beyond the seed coat; in soil by emergence of the greening cotyledons above the soil surface. The time sequence of imbibition, radicle protrusion, and radicle elongation concomitant with the greening of cotyledons has been previously investigated in detail (Walther 1981, 1982). In the present study employing saline solutions of various strength, this sequence was strictly monitored to distinguish between initial germination (radicle protrusion) and subsequent radicle elongation with greening of cotyledons, designated as 'transplantable seedlings'. The latter represent a developmental state comparable to the emergence of cotyledons above the soil surface.

For a given germination test in an aqueous solution, the percent of total germination and the percent of transplantable seedlings, based on the number of germinated seeds, were determined. In soil tests, the percent total germination and the time sequence of cotyledon emergence were monitored.

It is appropriate here to note discrepancies in the categorization of salinity stress. A 40 mM NaCl solution is equivalent to the conductivity of a soil solution exceeding 4 mS cm^{-1} and in plant physiological literature considered slightly saline. In some standard ecological references soils with conductivities from 0 to 4 mS cm^{-1} are categorized as nonsaline (Kreeb, 1974), while in some agronomic ratings soil solutions at 25 °C having a conductivity greater than 2 mS cm^{-1} are designated as slightly saline, 4 - 6 mS cm^{-1} as moderately saline and greater than 6 mS cm^{-1} as severely saline. In the present report, solutions of 20 to 40 mM NaCl and 10 to 20 mM Na_2SO_4 are considered to be slightly saline; 40 to 60 mM NaCl and 20 to 30 mM Na_2SO_4 moderately saline; and solutions exceeding 60 mM NaCl or 30 mM Na_2SO_4 severely saline. (Solutions exceeding 120 mM NaCl and 50 mM Na_2SO_4 exert excessive salinity stress).

The data presented in Table 1 encompass germination tests of seeds from 14 different sites (five of which are represented by two crop years) in aqueous NaCl or Na_2SO_4 solutions varying from slight

to severe salinity stress. The aqueous soil extract of approximately 17 mS cm^{-1} was prepared from a sulfate-rich soil of a known conductivity of 85 mS cm^{-1} (soil courtesy Mr. Don Gayton). Tests with fewer than three 20-seed replicates were due to insufficient numbers of intact seed, or limited incubator space and manpower.

The germinability of wild licorice seed is high for a wild plant species. A germination success approaching 50% is generally considered adequate for the maintenance and spread of a wild plant species by sexual reproduction (Baker, 1972). In this study, the percent total germination at moderate to severe salinities induced with NaCl or Na_2So_4 remained consistently high except for minor deviations from the respective controls. For instance, twice as many control than NaCl replicates were used for Liberty 1988, but the response by the Conquest 1987 seed indicates significant sensitivity to severe Na_2So_4 stress. Similarly, total germination in the sulfate-rich soil extract remained high but declined to less than 50% in two seed sources: Dafoe 1987 and Watrous 1987, both sites occur on soils with $MgSO_4$ as dominant salt.

On the basis of the percent total germination, the germinability of wild licorice seed, defined as radicle protrusion, is not impaired at even excessive salinity stress induced with NaCl or Na_2SO_4. However, a real indicator of successful germination sensu seedling establishment is represented by the percent of transplantable seedlings, shown in brackets in Table 1. In general, radicle protrusion occurred within two days and transplantable seedlings were scored on the fourth day from the start of imbibition, but at the higher salt concentrations a one-to-two day delay was common. Under the severe salinity stress radicle elongation was minimal (here ascribed to salinity - induced water stress) even in seedlings with fully greened cotyledons. Since the total number of seeds from a given source tested varied from about 400 to 3000, remarks here will be restricted to sources from which about 1000 or more seeds were tested.

The control seed source ‚Bell' suggested some v. iability between years. The plants were adequately watered during both

TABLE 1. Percent germination of Glycyrrhiza lepidota seed from different sources in aqueous sodium chloride or sodium sulfate at a 14 h photoperiod /25°C constant. In brackets, percent transplantable seedlings ($\bar{x}$ ± SD based on three or more 20-seed lots)[a]

	mM NaCl							mM Na_2SO_4							
CONTROL	20	40	60	80	100	120		10	20	30	40	50	60	70	Sulfate-Soil:Wat 1:5
							BELL 1987								
99±2 (91±11)	100 (77)	91 (86)	97 (80)	100 (79)	97 (68)	97 (50)		100±0 (80±10)[b]	100±0 (70±5)[c]	100±0 (66±8)[c]	98±3 (40±11)[c]	96±3 (38±7)[c]	96±6 (22±4)[c]	95±5 (6±3)[c]	92±3 (68±13)
							BELL 1988								
99±2 (93±4)	96±5 (81±7)[b]	98±2 (80±5)[c]	95±4 (61±12)[c]	97±3 (52±14)[c]	96±5 (55±8)[c]	93±2[b] (46±8)[c]		96±6 (94±11)	98±3 (94±9)	98±3 (88±10)	96±4 (79±14)	100±0 (67±17)[c]	95±8 (72±13)[c]	94±5 (47±9)[c]	97±3 (80±6)
							BESSANT 1987								
93±5 (81±15)	95±0 (83±5)	94±5 (83±17)	98±3 (79±5)	96±3 (77±11)	86±10 (65±20)	93±6 (62±23)		98±3 (73±8)	93±3 (54±19)[a]	98±3 (49±17)[a]	93±7 (24±9)[b]	91±8 (32±8)[b]	91±8 (12±12)[c]	96±3 (6±3)[c]	-
							CHAPLIN 1987								
96±6 (90±11)	80±18 (84±14)	78±21 (86±12)	80±27 (84±10)	79±23 (83±18)	89±13 (76±15)	87±11 (64±18)[a]		80 (60)	75 (55)	62 (31)	65 (27)	70 (50)	65 (21)	67 (27)	75 (61)
							CHAPLIN 1988								
94±6 (64±13)	-	-	-	-	-	-		91±6 (57±13)	90±7 (57±6)	90±8 (59±5)	86±6 (26±15)[c]	78±5[c] (37±12)[c]	85±4[c] (12±5)[c]	83±6[c] (16±8)[c]	-
							CONQUEST 1987								
88±5 (79±11)	60 (75)	55 (81)	50 (80)	55 (63)	75 (66)	75 (80)		60 (41)	50 (40)	65 (7)	30 (0)	40 (25)	35 (0)	35 (0)	55 (54)
							CYPRESS HILLS 1988								
92±5 (88±5)	92±3 (91±7)	93±7 (87±6)	92±8 (75±10)	92±3 (67±4)[a]	96±5 (64±14)[a]	91±3 (53±8)[a]		95±0 (89±0)	88±3 (74±2)[a]	95±5 (68±2)[a]	92±2 (53±2)[a]	88±3 (54±2)[a]	85±5 (61±1)[a]	90±5 (16±6)[a]	-
							DAFOE 1987								
83±11 (86±17)	83±8 (78±13)	70±5 (70±20)	86±6 (86±9)	81±10 (77±16)	75±13 (75±21)	73±16 (72±16)		95 (68)	85 (88)	90 (94)	95 (15)	95 (26)	90 (16)	95 (10)	43±40 (37±34
							LIBERTY 1987								
92±6 (86±17)	60 (80)	67 (100)	85 (37)	68 (100)	85 (0)	58 (100)		85 (55)	85 (58)	75 (40)	60 (8)	65 (38)	65 (7)	65 (0)	65 (69)
							LIBERTY 1988								
78±10 (88±8)	90±13[a] (73±14)[b]	90±10[b] (65±14)[c]	86±8[a] (48±23)[c]	85±14 (53±20)[c]	91±10[b] (54±21)[c]	82±24 (43±14)[c]		76±10 (80±13)[a]	78±10 (78±15)[a]	74±10 (69±19)[c]	74±13 (66±19)[c]	71±14 (50±18)[c]	76±10 (35±19)[c]	66±11 (25±13)[c]	70±5 (61±16
							OLD WIVES LAKE TWO-BAY PASTURE 1987								
62 (76)	92 (71)	84 (86)	92 (83)	92 (78)	92 (52)	76 (24)		70 (66)	45 (33)	40 (50)	60 (50)	80 (18)	55 (27)	25 (0)	69 (43)

CONTROL	mM NaCl						mM Na_2SO_4							Sulfate Solubility
	20	40	60	80	100	120	10	20	30	40	50	60	70	
OUTLOOK 1986														
92±10 (86±15)	86±14 (78±14)	88±10 (86±13)	89±12 (84±15)	87±10 (74±15)	79±25 (50±27)[c]	84±11 (50±26)[c]	86±10 (66±11)[a]	78±17 (71±8)	81±16 (56±23)[b]	73±23 (55±13)[a]	78±17 (40±10)[c]	61±33 (8±14)[c]	63±27[a] (15±18)[c]	75 (80)
PLAPOT 1986														
96±5 (94±5)	92±5 (98±3)	95±5 (95±5)	97±3 (100±0)	96±6 (89±6)	95±5 (87±10)	90±5 (70±17)[b]	100 (75)	100 (70)	100 (70)	100 (40)	100 (25)	100 (20)	90 (0)	85 (70)
PLAPOT 1988														
99±2 (83±7)	94±4 (70±10)	93±7 (61±20)	95±7 (60±20)[a]	95±4 (78±9)	92±5[a] (45±18)[c]	93±9 (45±3)[c]	95±4 (82±2)	92±7 (70±10)[b]	89±7[a] (74±3)[b]	98±2 (49±8)[c]	93±6[a] (39±24)[c]	78±12[c] (31±9)[c]	86±5[a] (19±22)[c]	-
STRAWBERRY LAKES 1987														
91±8 (84±4)	85 (86)	85 (65)	82 (75)	91 (79)	79 (44)	83 (26)	95 (68)	100 (60)	100 (75)	100 (55)	95 (21)	95 (21)	60 (33)	83±5 (28±1
STRAWBERRY LAKES 1988														
94±8 (76±15)	91 (85)	85 (70)	83 (78)	90 (80)	85 (45)	83 (35)	96±4 (73±14)	93±9 (68±12)	94±8 (59±11)[b]	97±3 (39±6)[c]	93±6 (35±10)[c]	90±11 (36±13)[c]	89±11 (24±7)[c]	86±1 (31±1
SWIFT CURRENT 1988														
100±0 (83±10)	98±3 (73±9)	98±3 (66±14)	96±3[a] (67±10)[b]	95±6[a] (63±13)[a]	97±4 (49±13)[c]	91±15 (48±15)[c]	100±0 (72±21)	97±3[a] (71±19)	99±1 (56±14)[c]	98±4 (49±9)[c]	96±5 (43±12)[c]	97±4 (33±8)[c]	97±4 (28±5)[c]	99±2 (48±1
WATROUS 1987														
95 (91)	70 (100)	95 (100)	75 (93)	75 (65)	80 (62)	80 (50)	100 (70)	100 (75)	90 (77)	85 (70)	95 (57)	70 (60)	-	35 (46)
WEST MAPLE CREEK 1981														
98±5 (93±6)	98±3 (69±19)[a]	95±5 (72±27)	96±5 (72±22)	94±4 (61±29)[a]	97±3 (80±20)	96±4 (60±40)	95 (52)	100 (65)	100 (75)	100 (35)	95 (26)	90 (22)	35 (0)	8 (4

[a] Values with different superscript letters are significantly different from the respective Controls: a at $P \leq .10$, b at $P \leq .05$, c at $P \leq .01$ (Mann-Whitney test).

growing seasons, thus, the hot summer of 1988 might be a contributing factor.

The percent of transplantable seedlings was significantly different ($P \leq .01$) relative to the water control from 40 mM NaCl onwards. But sensitivity to Na_2SO_4 is apparent only from 50 mM (severe salinity stress) onwards. The response of 1987 seed to Na_2SO_4 and sulfate-rich soil extract showed greater sensitivity to the sulfate ion. The Bell population originated from the West Maple Creek 1981 seed which in the present tests exhibited only minor sensitivity to NaCl (regrettably insufficient seed were available for more tests in aqueous Na_2SO_4), indicating a rather salt-tolerant physiotype which did not persist consistently in the progeny of the Bell population.

Of the field-collected seeds, those originating on plants with adequate moisture during the crucial periods of the growing season, the populations at Strawberry Lake and in the Cypress Hills are noted. Of the eight to ten replicates of the Strawberry Lake 1988 seed tested, sensitivity to Na_2SO_4 set in already at 30 mM with a consistent decline in the percent of transplantable seedlings. Since a similar decline was noted in the sulfate-rich soil extract, it is concluded that this seed source represents a physiotype more sensitive to the sulfate than to the chloride or sodium ions. However, the germination behavior of the Cypress Hills 1988 seed (from a population alongside a stream bank in the West Block) does not indicate moisture status during seed formation as influencing response to salinity stress since the Cypress Hills 1988 seed showed only very slight sensitivity to either NaCl or Na_2SO_4 differing from the control only at the 10% significance level.

Pertaining to seed sources from open-prairie populations: Bessant 1987 seed showed no sensitivity to NaCl but to Na_2SO_4. Regrettably the 1988 seed condition was poor, shrivelled and partly-developed seeds abounded. Since there was no apparent change in this large population relative to 1987, it is probable that the dry 1988 growing season accounted for the poor seed set. Attention is drawn to the Chaplin seed source originating from an extensive wild licorice stand subject to deposition of salt from

the lake. Although the percent of transplantable seedlings is relatively low even in the control, the stress imposed in these germination tests was minimal at moderate Na_2SO_4 salinity, suggesting this population contains salt-tolerant physiotypes. The greatest number of seed tested were those from the Liberty 1988 collection (courtesy Mr. Don Gayton). It showed a high sensitivity to moderate NaCl (and excessive Na_2SO_4) although the population occurs in an area naturally rich in NaCl. The Outlook 1986 seed was obtained from a pasture with obvious secondary salinization, and the seed is more sensitive to the high sulfate than to the high chloride ion concentration. A similar trend is evident in the Piapot seed source (again, 1987 seed condition was very poor), obtained from a large, persistent stand of wild licorice on stabilized sand. Of special interest is the behaviour of the Swift Current 1988 seed (again, courtesy Mr. Don Gayton). Nine to ten replicates were tested. All showed a depressing effect, relative to the control, at 100 and 120 mM NaCl, and from 30 mM Na_2SO_4 onwards.

On the basis of germination tests in aqueous solutions of increasing strength of NaCl or Na_2SO_4, and in part in an aqueous extract of a sulfate-rich soil, no seed source was highly salt-tolerant although various field-source seed showed less-to-greater sensitivity to moderate-to-severe salinity. However, comparison of the percent of transplantable seedlings indicates that most seed sources had a success near or in excess of 50% at moderate salinities, and some at severe salinities, suggesting that wild licorice could become established, in otherwise suitable habitat, at moderate soil salinities, which in southern Saskatchewan are imparted by sulfate salts, primarily.

Additional germination tests not shown in Table 1 were carried out at very high salt concentrations: 140 to 200 mM NaCl and 80 mM Na_2SO_4, as well as in sulfate-rich soil extracts of approximately 42 mS cm^{-1} . Radicle protrusion did not occur in the latter. In the 140-180 mM NaCl solutions germination was delayed by 1-to-2 days and only a very low percentage of transplantable seedlings

resulted, none at 200 mM. This suggests that for wild licorice seed the threshold tolerance to NaCl-induced salinity is attained near 120 mM, to Na_2SO_4 near 60 mM, thus, equivalent with regard to the sodium ion.

In Table 2 the germination behaviour of aged seed is given. The water-impermeable testa of wild licorice does not impart an embryo dormancy, and under field conditions the persistence of old seed ensures spread of the species to new sites. To the best of my knowledge, this is the first report on germination of aged seed of wild licorice. The seeds were kept in the laboratory, at room temperature, for 10 to 14 years. Relative to germination on water, the depressing effect of Na_2SO_4 was, again, greater than that of NaCl, on both the initial germination (radicle protrusion) and the development of transplantable seedlings, except for some seeds that originated in moist site conditions. The limited data suggest, however, that seed up to 14 years old can contribute to the colonization of field sites under mild-to-moderate salinity conditions, particularly in view of the fact that once established from seed, wild licorice reproduces successfully by vegetative means.

The germination behaviour of scarified seed in soil, on the basis of previous investigations, corresponds to that on wetted filter paper. As indicated in the progress report (February 1989), germination in a well-aerated, medium-textured loam was high (mean 80%, range 63-93%) in several seed sources tested. Mr. Don Gayton provided a fine-textured extremely saline soil (85 mS cm^{-1}, which was used for the aqueous soil extracts) which completely inhibited germination. This saline soil was diluted with the control soil. At approximately 2 and 7 mS cm^{-1} , 85 and 50% germination occurred but at 14 mS cm^{-1} only 5%. This low germination success is attributed in part to the loss of structure of the saline soil rather than solely to the salinity stress. A second lot of saline soil was also provided by Mr. D. Gayton. Again, this second lot was extremely fine-textured soil. It had a conductivity of 28.2 mS cm^{-1}

TABLE 1. Percent germination of Glycyrrhiza lepidota seed older than 10 years in aqueous NaCl and aqueous Na_2SO_4 at a 14h photoperiod/25°C constant. (In brackets, percent transplantable seedlings).*

Seed source	Control	mM NaCl						mM Na_2SO_4								Sulfate-rich Soil:H_2O 1:5
		20	40	60	80	100	120	10	20	30	40	50	60	70	80	
Avonlea 1975	70 (64)	70 (71)	85 (59)	55 (45)	35 (43)	55 (54)	40 (25)	45 (44)	45 (22)	40 (0)	25 (0)	30 (0)	20 (0)	25 (0)	-	47 (41)
Aylesbury 1974	60 (62)	80 (56)	75 (53)	95 (58)	45 (55)	75 (60)	80 (31)	-	-	55 (27)	-	25 (40)	-	-		37 (33)
Bogdan's Farm 1974	77 (49)	70 (54)	70 (67)	85 (70)	75 (67)	70 (28)	65 (28)	60 (33)	65 (7)	60 (8)	50 (0)	30 (0)	35 (0)	35 (0)	0	37 (5)
Caledonia 1974	70 (50)	70 (36)	75 (53)	65 (46)	65 (60)	55 (54)	40 (25)	-	-	-	-	-	-	-		50 (60)
Caledonia 1978	97 (100)	95 (95)	100 (85)	85 (82)	85 (82)	90 (50)	95 (11)	90 (83)	90 (66)	100 (75)	100 (40)	90 (16)	90 (0)	80 (0)	75 (0)	87 (42)
Cypress Hills, West Block 1974	15 (33)	5 (0)	5 (0)	5 (0)	-	-		-	-	-	-	-	-	-	-	-
Good Spirit Lake 1975	50 (51)	50 (70)	30 (100)	50 (90)	40 (100)	40 (100)	35 (43)	30 (0)	20 (0)	20 (30)	25 (0)	15 (33)	0	0	0	0
Great Sand Hills 1975	82 (71)	75 (60)	65 (53)	90 (58)	80 (50)	95 (42)	55 (9)	75 (93)	80 (62)	65 (15)	90 (55)	65 (23)	45 (11)	20 (25)	20 (25)	55 (37)
Piapot 1974	80 (77)	50 (30)	55 (45)	50 (30)	55 (30)	60 (23)	35 (0)	80 (56)	80 (68)	55 (63)	55 (18)	50 (10)	25 (0)	15 (0)	15 (0)	27 (38)
Piapot 1978	90 (71)	65 (45)	80 (56)	80 (50)	75 (20)	50 (20)	55 (9)	-	-	35 (20)	-	35 (28)	-	-	-	57 (10)
Saskatchewan Beach 1974	87 (77)	95 (74)	95 (68)	85 (70)	80 (44)	85 (29)	70 (29)	60 (50)	70 (42)	45 (44)	40 (12)	55 (27)	35 (14)	15 (0)	25 (0)	77 (39)

*Due to limited seed stock, based on 20-seed lots except Control and 1:5 soil extract.

was enriched with the sulfate ion, and a pH of 8.6 (analysis by the Saskatchewan Soil Testing Laboratory). It was mixed with the control soil to obtain approximately 2, 4, 7 and 14 mS cm^{-1}. A soil:water slurry 1:1 gave pH reading: control 6.7; 2, 4, 7 and 14 mS cm^{-1} a pH of 7.2, 7.5, 7.6 and 7.9 respectively. These soil mixtures were used for germination tests in growth chambers and out-of-doors (tap water was used for daily watering). The in-soil-germination data are given Table 3. Five different seed sources were tested. In the undiluted saline soil (28.2 mS cm^{-1}) the few one seed/pot seedlings appeared only in the growth chambers after considerable delay, relative the lower salinities, and two survived to this date. The green cotyledons showed only minimal expansion and the excavated radicle showed typical water-logged conditions. Again, a synergistic inhibitory effect is here ascribed to the lack of soil structure causing water-logged conditions in addition to the salinity stress. Similarly, germination in the 14 mS cm^{-1} soil mixtures was low, both in the growth chamber and out-of-doors (except Bell 1988). The emergence of seedlings was consistently delayed from 3 to 7 days, and survival of seedlings was low, although in some pots one-two seedlings survived exhibiting much slower growth (at present a few 4-month old seedlings, in a growth chamber, are sturdy, showing no deviation from the normal morphology of young wild licorice plants but are much smaller relative to the control and lower salinity seedlings).

In general, the germination success in the 2 to 7 mS cm^{-1} soil mixtures was comparable, although a slight delay in emergence was noted in the 7 ms cm^{-1} soil. Under the growth chamber conditions, the highest germination and seedling survival was observed in the Swift Current 1988 seed source. In the out-of-doors trials all four seed sources showed higher percent total germination and seedling survival than under the growth chamber conditions. The out-of-door germination success and seedling survival in soil of moderate salinity stress (4 mS cm^{-1}) suggest a common threshold tolerance limit to salinity stress, under field conditions, in view of the decline in the 7 mS cm^{-1} soil mixtures in the three field-seed sources. It is of interest to compare the observed

TABLE 3. Percent germination of Glycyrrhiza lepidota seed in saline soil mixtures. ($\bar{x}$ ± SD based on three or more 20-seed lots; in brackets range).

Approximate Soil Salinity					
Control	2mS cm^{-1}	4mS cm^{-1}	7mS cm^{-1}	14mS cm^{-1}	28mS cm^{-1}
A. 14h photoperiod 25°C/10h 15°C (growth chamber)					
		Bell 1988			
83±16 (65–95)	63±20 (40–75)	75±15 (60–90)	66±6 (60–70)	30±5 (25–30)	0
		Cypress Hills 1988			
88±8 (80–95)	56±10 (45–65)	68±17 (50–85)	61±10 (50–70)	50±9 (45–60)	5±0 (5–5)
		Liberty 1988			
70±18 (55–90)	58±21 (35–75)	53±10 (45–65)	43±21 (20–60)	38±11 (25–45)	5±0 (5–5)
		Strawberry Lake 1988			
95±0 (95–95)	90±5 (85–95)	60±10 (50–70)	53±25 (25±75)	26±19 (5–40)	0
		Swift Current 1988			
85±10 (75–95)	90±10 (80–100)	76±10 (65–85)	63±13 (50–75)	35±30 (15–70)	0
B. Out-of-doors Mid-summer 1989					
		Bell 1988			
98±3 (95–100)	86±10 (75–95)	80±17 (70–100)	86±6 (80–90)	70±0 (70–70)	0
		Liberty 1988			
80±5 (75–85)	65±17 (45–75)	61±8 (55–70)	53±11 (40–60)	25±9 (20–35)	0
		Strawberry Lake 1988			
96±3 (95–100)	86±8 (80–95)	73±15 (60–90)	53±13 (40–65)	33±10 (25–45)	0
		Swift Current 1988			
86±9 (75–100)	85±8 (75–95)	70±8 (60–85)	37±6 (30–45)	7±9 (0–25)	0

germination behaviour of wild licorice (a native legume) with other leguminous plants. For instance, germination of peanut was reported to be less sensitive to salinity stress than seedling development (Shalhevet et al., 1973, as cited by Meiri, 1984), and Waissman & Miyamoto (1987) reported that the germination of two alfalfa cultivars declined at salinities in excess of 4.3 mS cm^{-1} . Attention is drawn to the germination behaviour of the Swift Current 1988 seed lot which clearly indicate the effect of severe soil salinity stress (here about 7 mS cm^{-1}, enriched with the sulfate ion) parallels the stress imposed by aqueous Na_2SO_4.

GROWTH RESPONSE OF WILD LICORICE TO SALINITY STRESS

The transplantable seedlings resulting from germination in aqueous solutions were hand-transplanted into and raised in vermiculite irrigated with 1/2-strenght nutrient solution made to the desired strength with NaCl or Na_2SO_4, or to approximately 17 mS cm^{-1} with an indiluted sulfate-rich soil extract. The pH of the NaCl solution (20 to 120 mM) ranged from 6.8 to 5.7; that of the Na_2SO_4 solution (20 to 60 mM) from 6.3 to 6.8, and nutrient solution with the soil extract had a pH of about 6.4 (the pH of different batches of double-distilled water fluctuates). The 1/2-strength nutrient solution was made full-strength with respect to iron since preliminary growth experiments suggested incipient iron deficiency even in the control. That salinity stress results in decreased absorption of magnesium and iron, required for chlorophyll synthesis, has been reported (for instance by Reddy, 1967, as cited in Malakondaiah and Rajeswararao, 1980). The seedlings were kept in growth chambers, differing somewhat in the incident light intensity, under a 14 h photoperiod with alternating day/night temperatures of 25/15 °C.

The normal development of wild licorice seedlings shows a morphological sequence form photosynthetic cotyledons to true foliage leaves that are simple at nodes 1 and 2. The first

compound leaves are trifoliate and generally appear at node 3 and upwards as the seedling stem elongates. The number of nodes bearing trifoliate leaves varied even in the controls under the standard growing conditions. Pentafoliate, and rarely tetrafoliate, leaves appeared at the upper nodes but typical multi-leaflet (seven or more) compound leaves did not develop on the primary (initial) seedling stems. Multi-leaflet leaves developed on the experimental plants only on secondary stems originating from adventitious buds, generally in seedlings older than 5 wk. Under the given standard growing conditions, the simple and first trifoliate leaves did not persist for long but their photosynthetic contributions to the growth of the early seedlings was paramount.

The growth and development was comparable to the controls at mild and moderate salinity stresses but seedling survival declined sharply from 80 mM NaCl and 40 mM Na_2SO_4 onwards in most seed source populations. The common symptom of salinity stress was the deterioration of the seedling root system. No morphological deviations in the shoot were, however, observed at the high salt concentrations, only general retardation of growth.

The initial growth of wild licorice postgerminative seedlings is supported by the proteinaceous reserves in the seed, and by the ensuing photosynthesis by the green cotyledons. For this reason, cotyledon chlorophyll was determined in selected seed source populations after 1 to 3 wk from transplanting into vermiculite. Normal cotyledon senescence, in the control, generally set in by the end of the 4th week. No acceleration in the onset of senescence in salt-stressed cotyledons was observed in the present study (Reference is here also made to the progress report February 1989).

The slightly depressing effect of NaCl on cotyledon chlorophyll is already apparent in 2 wk seedlings as shown in the scatterdiagram (Fig. 1) for three 1987 seed source populations. More pronounced was the effect of Na_2So_4 stress on both cotyledon expansion (expressed as cotyledon mass, mg fresh weight) and total chlorophyll content per cotyledon. Representative data are given

for Liberty 1988 (Fig. 2) and Swift Current 1988 (Fig 3) seed source populations. All seedlings of Liberty survived into the 4th week although later succumbed to severe salinity stress. The decline in cotyledon chlorophyll, and reduced size, was progressive but in the sulfate-rich soil extract the chlorophyll was less affected, being equivalent to 40 mM Na_2SO_4 although cotyledon size decreased more. By contrast, the effect of Na_2SO_4-induced salinity was greater in Swift Current seedlings, and insufficient seedlings survived into the third week in 40 mM. The suppression is already evident at 10 mM Na_2SO_4 (mild salinity stress). The Swift Current 1988 seed source also showed greater sensitivity to Na_2SO_4 in the germination behaviour, and coupled with the decline in cotyledon size and chlorophyll synthesis, this seed source emerges as the least salt-tolerant physiotype in the early stages of seedling growth. Although this seed originated on a site showing signs of secondary salinization, it is doubtful that this seed source would lead to colonization of field sites with moderate Na_2SO_4, or similar sulfate salts, soil salinity.

The interest in salinity stress on chlorophyll is generally centered on leaf chlorophyll but Masih et al. (1983) studied cotyledon chlorophyll synthesis in nine cultivars of Brassica campestris under increasing salinity from 4 to 16 mS cm^{-1}. They reported enhanced chlorophyll accumulation, peaking at 12 mS cm^{-1} in some cultivars. In the scatterdiagrams (Figs. 1 and 2) there is apparent a slight enhancement when total chlorophyll is expressed per gram cotyledon tissue, however, when expressed as chlorophyll per organ, it is evident (Figs. 2 and 3) that increasing salinity stress has a consistent depressing effect on cotyledon chlorophyll, particularly in 2 wk cotyledons. Since the shoot apex with the primordia of the first foliage leaves emerged by the end of the first and beginning of the second week, after transplanting under the standard growing conditions, reduced cotyledon chlorophyll was of great physiological significance to the growing seedlings.

The effect of NaCl-induced stress on simple and first trifoliate leaves of 5 wk seedlings is evident in Fig. 4 (both 1987 seed). The Strawberry Lake stands of wild licorice occurred on

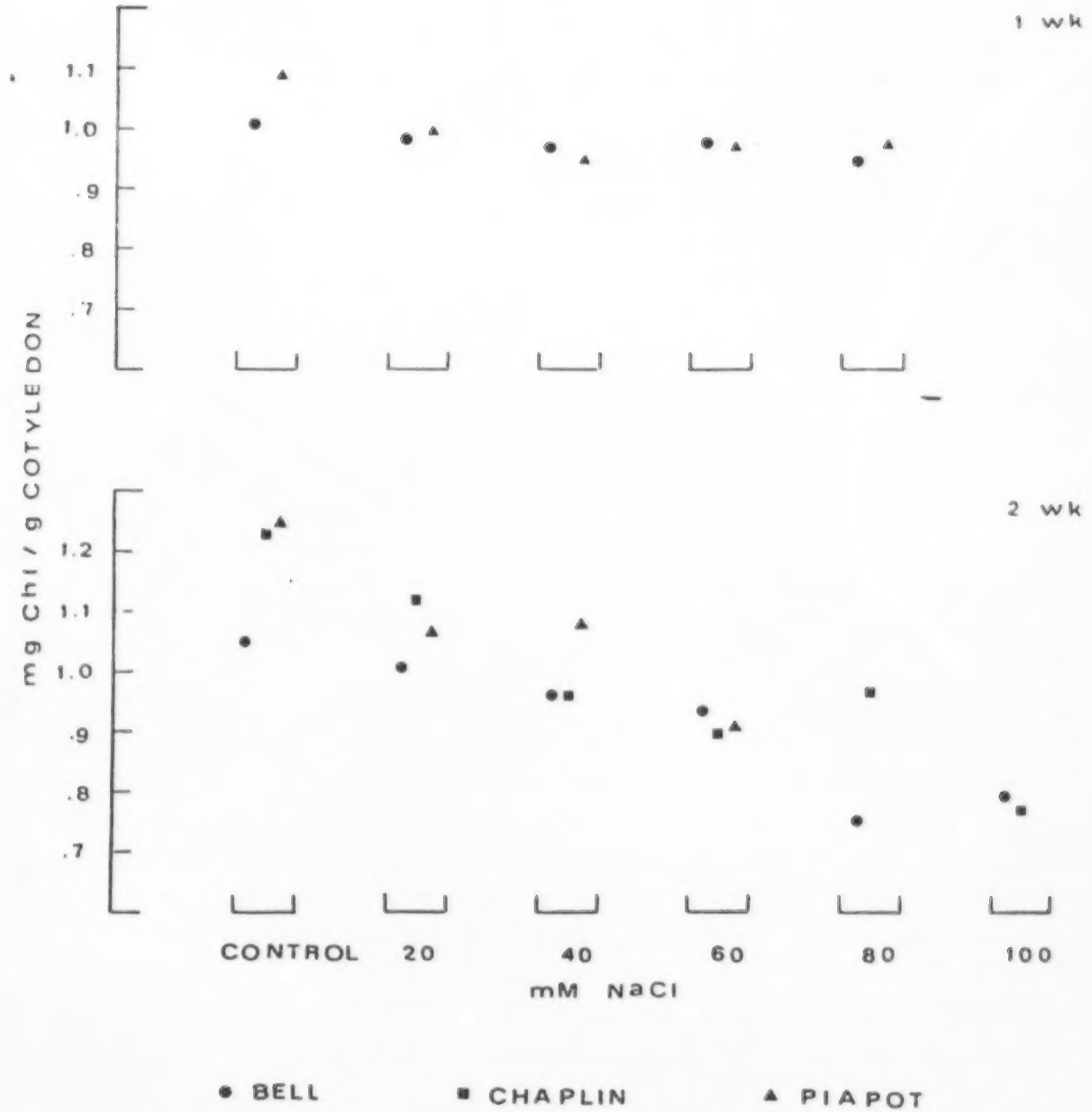

FIG. 1. Effect of NaCl-induced salinity on cotyledon chlorophyll content of 1- and 2-week seedlings of wild licorice grown under the described standard conditions. Seed sources : Bell 1987, Chaplin 1987 and Piapot 1986.

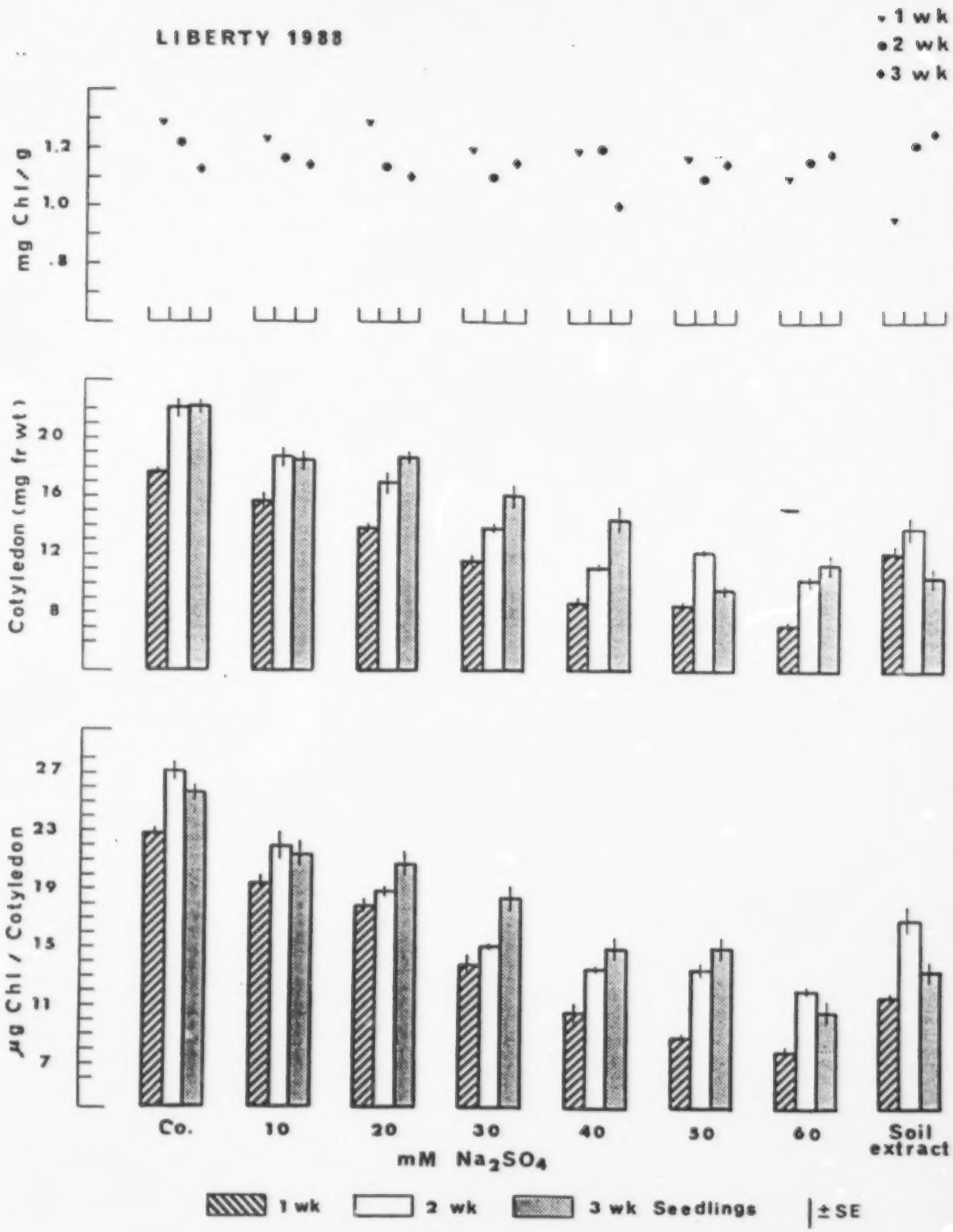

FIG. 2. Effect of Na_2SO_4-induced salinity and a sulfate-rich soil extract of 17 mS cm^{-1} on cotyledons of 1-, 2- and 3-week seedlings of wild licorice grown under the described standard conditions. In the scattergram is shown mg total Chl per g fr wt, in the histograms cotyledon mass and total Chl per cotyledon. Seed source : Liberty 1988.

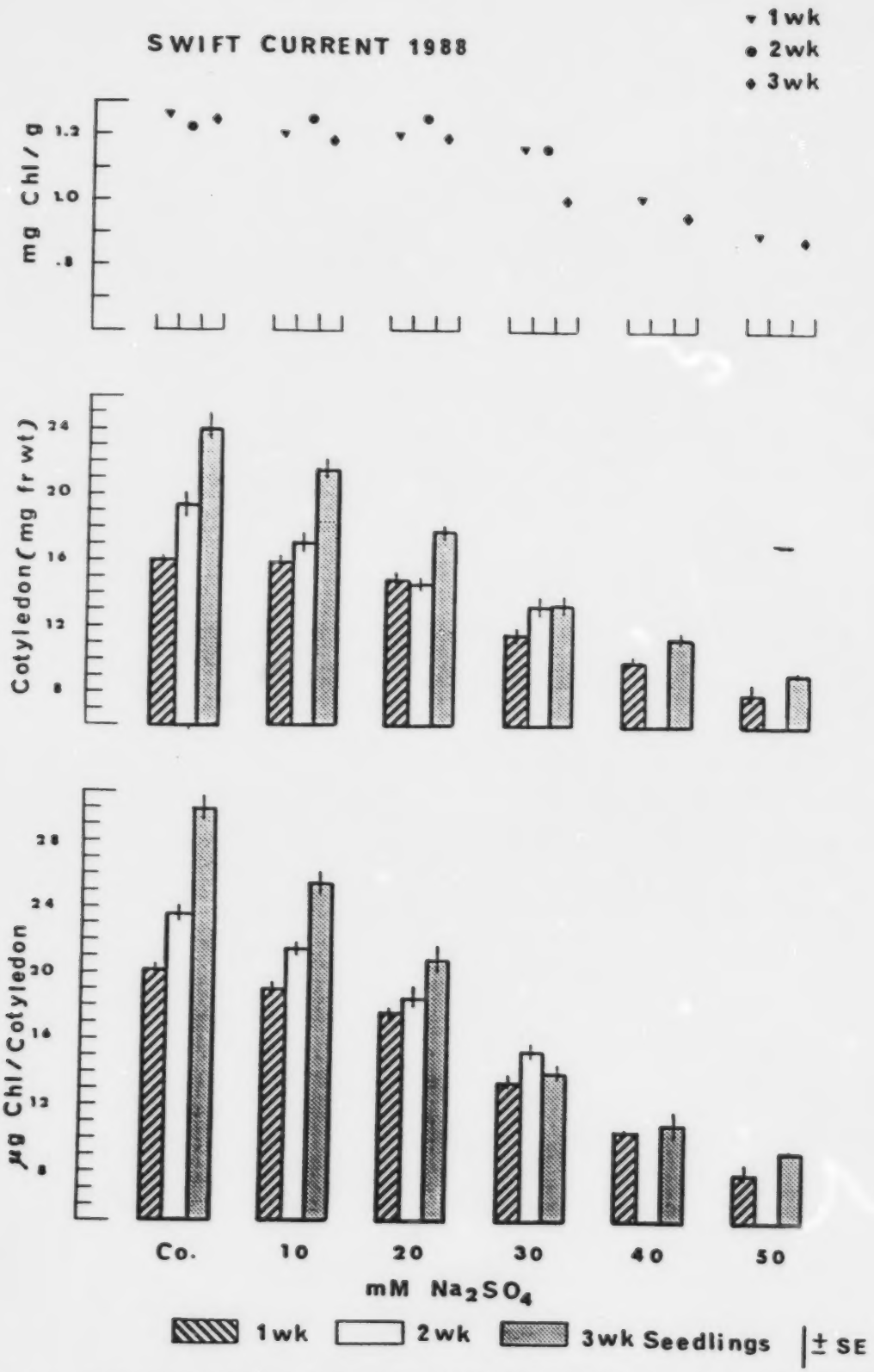

FIG. 3. Effect of Na_2SO_4-induced salinity on cotyledons of 1-, 2- and 3-week wild licorice seedlings grown under the described standard conditions. In the scatterdiagram is shown mg total Chl per g fr wt, in the histograms cotyledon mass and total Chl per cotyledon. Seed Source : Swift Current 1988.

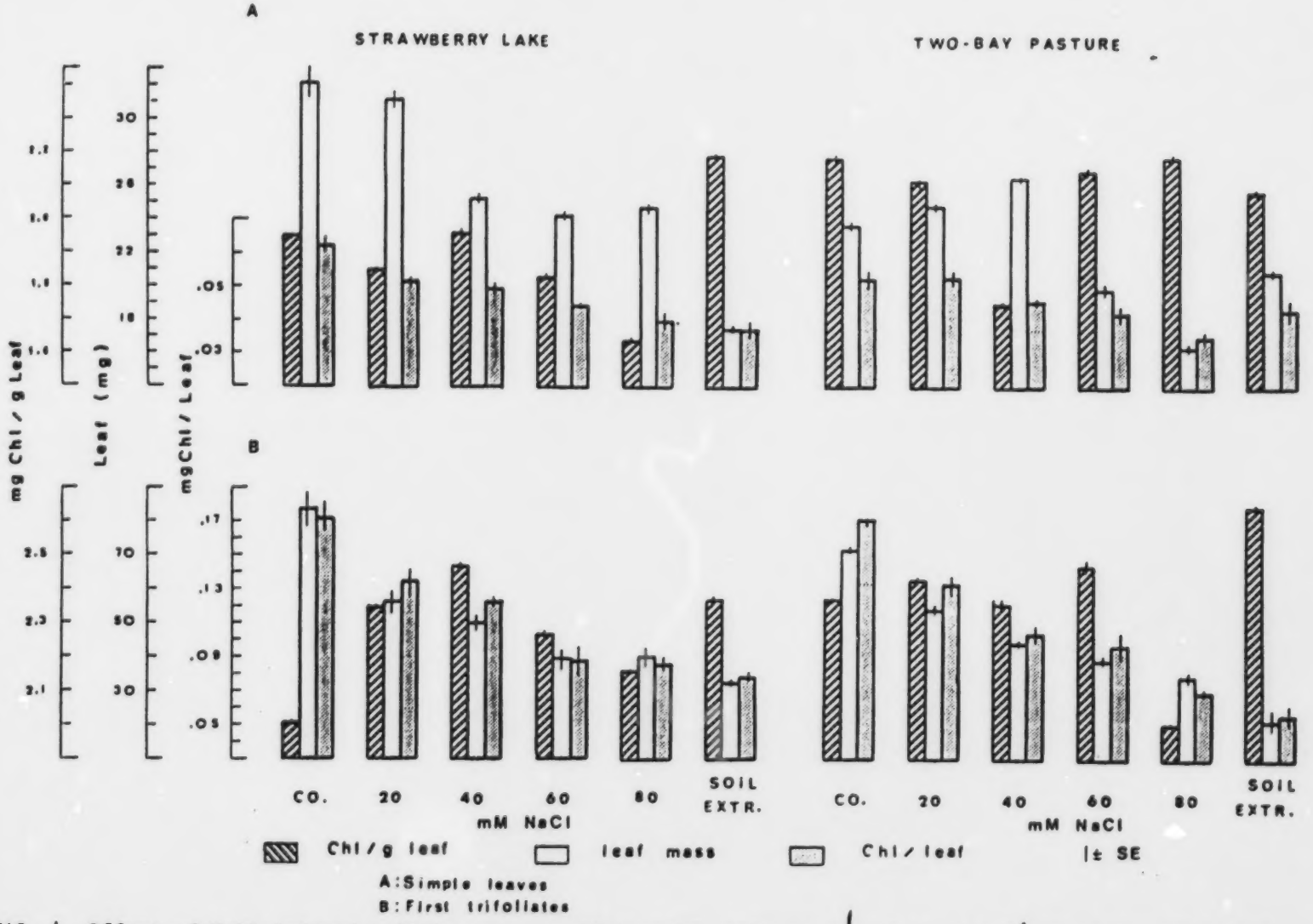

FIG. 4. Effect of NaCl-induced salinity and a sulfate-rich soil extract of 17 mS cm^{-1} on leaves of 3-week wild licorice seedlings grown under the described standard conditions. Shown are mg total Chl per g fr wt, leaf mass in mg fr wt and total Chl per simple leaf (at nodes 1 and 2), and first trifoliate leaf. Seed sources : Strawberry Lake 1987 and Two-Bay Pasture 1987.

relatively moist sites, while the fairly large Two-Bay Pasture population was near the Old Wives Lake and subject to salt deposition from the lake (it is reiterated here that the yield of intact seed was extremely low in this collection). Attention is drawn to leaf size (expressed as mg fresh weight) and total chlorophyll per organ. In simple leaves, chlorophyll declined slowly but steadily in both populations, however, the size of simple leaves declined sharply at 60 mM NaCl (designated as the upper limit of moderate salinity stress) in the Two-Bay Pasture seedlings. The trend is similar in the first trifoliate leaves, and again, leaf size was affected more in the Two-Bay Pasture seedlings. It must be noted that the smaller leaves generally appeared a darker green which is reflected in the total chlorophyll content when expressed per gram leaf tissue. However, the reduced leaf surface area translates into decreased photosynthesis, reflecting the reduced chlorophyll content expressed per organ.

The response to both NaCl- and Na_2So_4 induced salinity stresses is illustrated by 3 wk seedlings derived from the Swift Current 1988 seed source (Fig. 5). The 40 mM seedlings were stunted and succumbed to the continuing stress a week later. The effect on chlorophyll per organ is particularly evident in the first trifoliate leaves, NaCl and Na_2SO_4 exerting near-equal suppression. Since the first compound leaves are a major source of photosynthate for the growing seedlings, the decrease in chlorophyll is a reflection of lower photosynthesis. The depressing effect of salinity on chlorophyll synthesis has been reported for a variety of plants: Divate and Pandey (1981) in grape cultivars stressed with both Na_2SO_4 and $MgCl_2$; El-Sharkawi et al. (1986) in cotton, rama, and millet leaves; Chavan and Karadge 91986) in Sesbania grandiflora (a legume) which exhibited luxurious growth up to 10 mS cm^{-1}. However, with regard to changes in the chlorophyll a/b ratios the evidence is less definitive. In the aforementioned grape cultivar leaves changes in the Chl a/b ratios relative to the controls was mainly due to reduced synthesis of Chl b; in Sesbania Chl b was slightly reduced at the lower salinities but Chl a/b ratios equivalent to the control occurred at

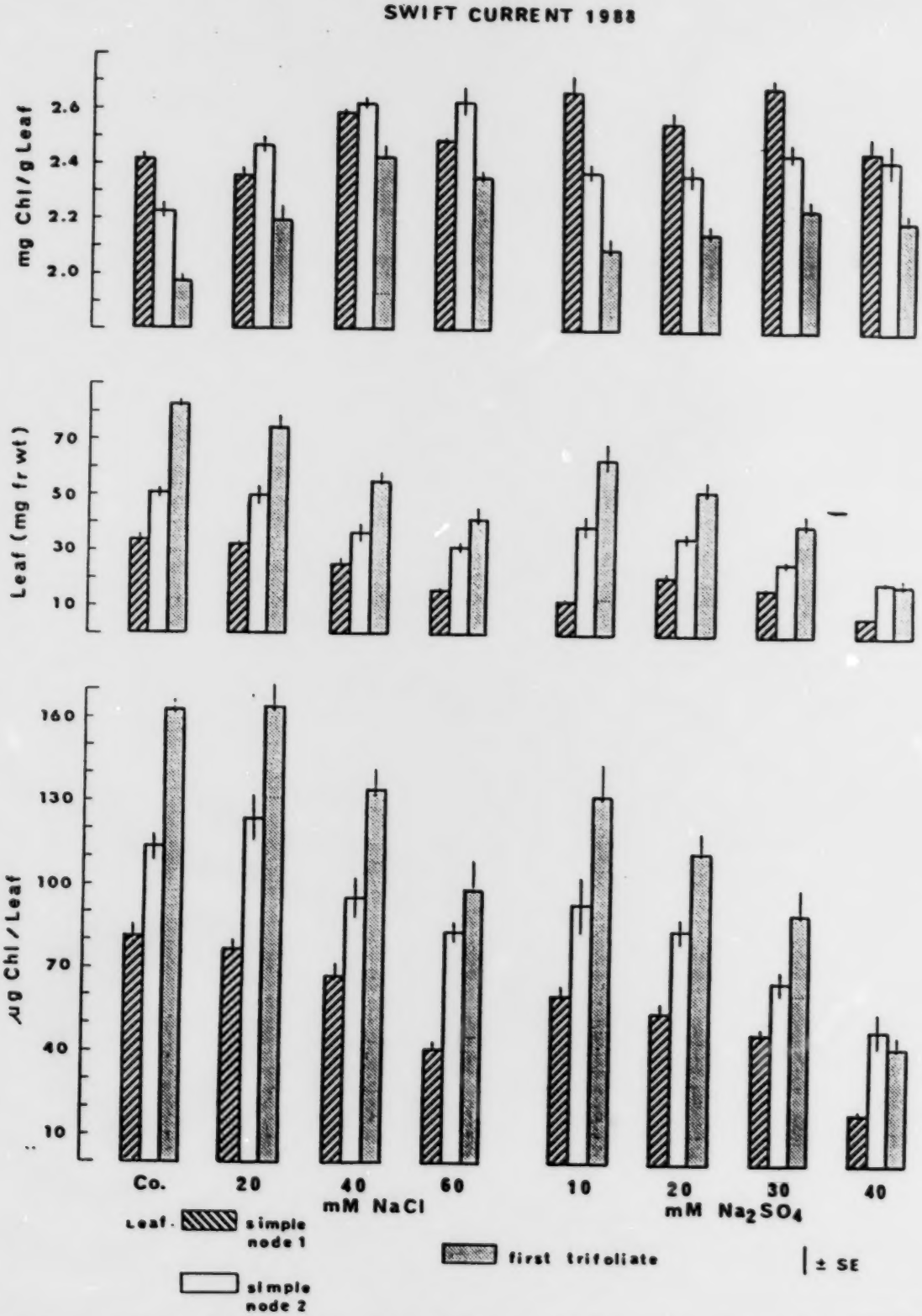

FIG. 5. Effect of NaCl- and Na_2SO_4-induced salinity on leaves of 3-week old wild licorice seedlings grown under the described standard conditions. Shown are mg total Chl per g fr wt, leaf mass and total Chl per simple leaf at node 1 and at node 2, and first trifoliate leaf. Seed source : Swift Current 1988.

the high salinities. Robinson et al (1983) found no change in the Chl a/b ratios in spinach leaves subjected to high NaCl-induced stress. In the present study, both cotyledon and leaf Chl a/b ratios showed minor changes relative to the respective controls, but the trends were inconsistent as exemplified for leaves in Table 5. The physiological consequences of reduced levels of Chl b, relative to Chl a, are complex, but the influence on carbohydrate metabolism could impair the deposition of reserves in the taproots of wild licorice seedlings (see below).

The effect of salinity stress on the growth of plants is customarily assessed by changes in accumulated phytomass and/or yield for crop plants. (Pertinent studies are cited in Marschner, 1986). In this investigation special attention was given to the deposition of reserves in the primary root of the seedlings which enlarge into a taproot. The reserves are primarily carbohydrate (e.g. the 'sweet'roots of wild licorice are already present in young seedlings, even at moderate-to-high NaCl concentrations). These reserves support the development of adventitious buds, at the junction of the cotyledonary node and upper root surface, from which arise the secondary shoots that consistently replaced the initial seedling shoots under the standard growing conditions, both in the controls and salt-stressed seedlings. The formation of such adventitious buds was not inhibited at moderate-to-severe salinity induced with either NaCl or Na_2SO_4 . Excessive salt concentration seedlings generally did not survive long enough for adventitious bud formation. The time of onset and the number of adventitious bud per individual seedling was, however, influenced by the severity of stress. In the control and light-salinity seedlings the formation of adventitious buds ensued as early as the 5th week, after transplanting, and progressively increased. In 8 to 9 wk seedlings even in the presence of 100 mM Nacl or 50 mM Na_2SO_4 at least two or more small adventitious buds appeared. Since the formation of adventitious buds closely parallels the enlargement of the primary seedling root, the latter was chosen as a parameter of

TABLE 4. Effect of salinity on Glycyrrhiza lepidota seedlings grown in vermiculite irrigated with 1/2-strength nutrient solution with added NaCl or Na_2SO_4 under a 14h photoperiod 25°C/10h 15°C. (X ± SE)*

MEASUREMENTS:	CONTROL	20	40	60	80	100
			mM NaCl			
		LIBERTY 1988: 8 wk Seedlings				
Stem height (cm)	13.7±.9	14.7±.9	10.5±.4[b]	8.4±.3[c]	5.9±.5[c]	3.8±3[c]
Dry wt trifoliate leaf (mg)	48.4±3.6	53.3±4.1	44.1±3.8	34.3±2.7[b]	23.1±1.3[c]	14.9±1.6[c]
Dry wt pentafoliate leaf (mg)	57.2±3.8	56.7±3.6	56.5±6.7	45.6±4.1[b]	25.7	23.0
X dry wt primary root	40.3±.9	39.6±.6	40.6±1.1	41.9±.8	38.4±1.1	39.6±1.4
		LIBERTY 1988: 9 wk Seedlings				
Stem height (cm)	16.7±.8	14.6±.5	10.0±.3[c]	8.2±.3[c]	7.9±.8[c]	4.4±.4[c]
Dry wt trifoliate leaf (mg)	44.1±2.7	46.7±3.3	38.0±2.0	31.9±1.9[c]	30.9±4.0[b]	22.1±1.7[a]
Dry wt pentafoliate leaf (mg)	51.1±3.6	49.8±3.5	42.8±2.7	41.7	49.1±4.1	34.2
X dry wt primary root	40.2±.7	40.0±.6	43.0±.7[c]	43.0±1.0[a]	33.0±.9[c]	37.8±2.2
		STRAWBERRY LAKE 1988: 9 wk Seedlings				
Stem height (cm)	16.9±.7	14.2±.5[b]	11.6±.6[c]	11.0±.5[c]	8.0±.2[c]	-
Dry wt trifoliate leaf (mg)	37.4±2.2	38.4±3.1	33.3±3.8	30.2	21.5±1.5[c]	-
Dry wt pentafoliate leaf (mg)	39.5±2.5	36.9±2.3	39.6±3.3	34.5±2.4	24.3±1.9[c]	-
X dry wt primary root	36.6±.6	38.6±.6[c]	37.8±.9[c]	35.0±.8	35.9±.8[b]	-
		SWIFT CURRENT 1988: 8 wk Seedlings				
Stem height (cm)	11.9±.5	10.8±.4	10.0±.5[b]	8.0±.2[c]	5.6±.5[c]	3.1±.3[c]
Dry wt trifoliate leaf (mg)	37.8±2.8	34.7±2.0	26.5±1.4[c]	23.2±.9[c]	20.5±1.7[b]	18.3±2.8[c]
Dry wt pentafoliate leaf (mg)	43.7±2.1	45.4±3.2	41.1±2.9	32.6±3.4[a]	34.3±2.6[b]	-
X dry wt primary root	36.0±1.2	38.7±.7	36.2±1.1	36.4±1.1	34.5±1.1	33.6±2.9
			mM Na_2SO_4			
	Control	10	20	30	40	50
		LIBERTY 1988: 8 wk Seedlings				
Stem height (cm)	11.9±.5	10.8±.3[a]	8.4±.3[c]	6.7±.3[c]	6.0±.4[c]	5.0±.3[c]
Dry wt trifoliate leaf (mg)	46.0±2.0	44.0±2.9	35.1±2.2[b]	34.2±1.9[c]	25.1±1.5[c]	31.0±4.3[b]
Dry wt pentafoliate leaf (mg)	50.1±2.0	42.2±2.8[b]	39.8±2.0[c]	40.0±2.1[c]	40.2±5.3[c]	45.3
X dry wt primary root	40.3±.5	43.0±.6[c]	45.2±.6[c]	44.2±.5[c]	41.8±.8	37.6±1.2
		LIBERTY 1988: 9 wk Seedlings**				
Stem height (cm)	11.0±.8	10.2±.6	7.0±.3[c]	7.4±.4[c]	8.0±.1[c]	-
Dry wt trifoliate leaf (mg)	47.1±4.1	42.9±3.1	36.4±3.4	36.9±2.5	30.6±2.8[a]	-
Dry wt pentafoliate leaf (mg)	64.5±5.1	59.9±6.1	55.4	-	55.2±3.1	-
X dry wt primary root	45.1±.8	44.9±.9	45.7±.7	46.2±.7	36.7±2.6[a]	-

Continued....2

	mM Na_2SO_4					
	Control	10	20	30	40	50
	STRAWBERRY LAKE: 9 wk Seedlings					
Stem height (cm)	17.5±.7	16.7±1.0	16.7±1.2	10.9±.4[c]	13.3±.9[c]	-
Dry wt trifoliate leaf (mg)	51.2±1.9	46.9±2.4	45.1±2.2[a]	44.8±3.5	48.2±4.4	-
Dry wt pentafoliate leaf (mg)	61.1±2.5	56.5±4.9	54.9±8.0	57.3±3.4	70.6±6.7	-
% dry wt primary root	42.1±.3	42.1±.9	42.2±.7	42.8±.8	38.1±1.9[a]	-
	SWIFT CURRENT 1988: 8 wk Seedlings					
Stem height (cm)	11.8±.3	13.6±.5[a]	11.3±.5	9.6±.5[c]	7.9±.4[c]	-
Dry wt trifoliate leaf (mg)	34.8±1.6	37.5±5.6	31.6±1.7	36.2±1.8	23.4±2.1	
Dry wt pentafoliate leaf (mg)	42.1±2.5	57.7±5.2[a]	47.5±4.1	47.4±3.2	37.4	-
% dry wt primary root	37.9±.6	37.3±.5	37.7±1.1	37.6±.9	29.4±.9[c]	-

* Values with superscript letters are significantly different from the respective Controls: a at P ≤ .10, b at P ≤ .05, c at P ≤ .01 (Mann-Whitney test).

** Different growth chamber with 25% lower light intensity under the given photoperiod and temperature regime.

TABLE 5. Leaf Chl a/b ratios ($\bar{X}$ ± SE) of 3 wk seedlings of *Glycyrrhiza lepidota* (seed source shown below).

Treatment	Leaf: Simple, node 1	Simple, node 2	First trifoliat
	LIBERTY 1988		
Control	2.8 ± .05	2.8 ± .04	2.8 ± .04
10 mM Na_2SO_4	2.7 ± .04**	2.8 ± .03	2.8 ± .03
20 mM	2.7 ± .06	2.8 ± .02	2.9 ± .05
30 mM	2.7 ± .06	2.8 ± .03	2.9 ± .06
	STRAWBERRY LAKE 1988		
Control	2.7 ± .04	-	2.9 ± .04
10 mM Na_2SO_4	2.6 ± .02***	-	2.8 ± .02
20 mM	2.6 ± .03	-	2.7 ± .02**
30 mM	2.7 ± .03	-	2.8 ± .03***
	SWIFT CURRENT 1988		
Control	2.8 ± .01	2.8 ± .03	2.7 ± .03
10 mM Na_2SO_4	2.7 ± .02	2.8 ± .02	2.8 ± .03**
20 mM	2.8 ± .02	2.8 ± .02	2.9 ± .03*
30 mM	2.7 ± .02	2.8 ± .02	2.9 ± .05**
40 mM	2.7 ± .01	2.7 ± .02	2.7 ± .03
20 mM NaCl	2.8 ± .02	2.9 ± .05	2.8 ± .04*
40 mM	2.8 ± .03*	2.9 ± .02*	2.9 ± .03***
60 mM	2.9 ± .02**	2.9 ± .02	2.9 ± .04***

Differ from Control:
* at 0.10, ** at 0.05 and *** at 0.01 level of significance

growth, under the standard growing conditions in this investigation. (It is noted that seedlings grown in the various saline soil mixtures did not deviate from this developmental pattern, as evidenced by the appearance of secondary shoots in some seedlings surviving even at 14 mS cm^{-1}).

For a comparative assessment of seedling growth under the induced salinity stresses, the main parameters measured were stem height (from the cotyledonary node to the apex), weights of shoots, selected foliage leaves and primary roots (stripped of the fine feeding roots). Since the weights of primary roots of the controls and light-salinity seedlings differed from the taproots of seedlings at higher salinities, the percent dry weight was chosen as a more consistent parameter for comparisons (percent dry weights, determined at the same drying conditions, allow for comparison of dry matter regardless of the water content of various tissues).

In Table 4 are given representative data of growth parameters measured for 8 and 9 wk seedlings of Liberty, Strawberry Lake and Swift Current (all 1988 seed) under increasing salinity stress induced by both NaCl and Na_2SO_4. Seedling height was significantly suppressed in all three seed source populations from moderate salinity onwards, with the exception of 9 wk Strawberry Lake and 8 wk Swift Current seedlings in which stem elongation was significantly affected only at 30 mM and 40 mM Na_2SO_4. Mature trifoliate leaves generally remained at nodes 4, 5, while mature pentafoliate leaves tended to occur at nodes 5 and 7. The size of these leaves varied in surface area, length of petioles and mass. The leaf data represents the average dry weight per leaf in mg. A significant depressing effect by NaCl on trifoliate leaves set in at 60 mM and greater, except in the Swift Current seedlings at 40 mM NaCl already (designated as the upper limit of mild salinity stress). Sodium sulfate generally exerted less stress on trifoliate leaves, but again, the response by seedlings from different seed sources can vary as indicated by 9 wk Liberty seedlings. Noteworthy is the 'relative resistance' of the

trifoliate leaves of the Swift Current population, suggesting that young seedlings could grow (albeit at a slower rate) under slightly saline conditions. As expected, the average dry weights of pentafoliate leaves is greater than that of trifoliate ones but it must be noted that individual differences in the leaflets (fully expanded surface area) were common. On the basis of the pentafoliate leaf mass, accumulated under controlled standard growing conditions, the salinity stress is ameliorated in the leaves at higher nodes, indicating again that salinity stress is most severe in the youngest seedling stages. Noteworthy is the response of the 8 wk Liberty pentafoliate leaves that are significantly smaller already at mild sodium sulfate stress unlike those of the older, 9 wk seedlings raised under lower light intensity. This suggests a synergistic effect of the light regime, as would occur under field conditions. By contrast, 9 wk Strawberry Lake pentafoliate leaves appear unaffected by Na_2SO_4 differing from the control only at 10% significance level in the 40 mM solution. An apparent enhancement of the pentafoliate leaf sizes at low NaCl and Na_2SO_4 was also noted in other seedling populations not reported in Table 4, suggesting that slightly saline soil conditions may favour the growth of wild licorice (further research under field conditions would be desirable).

The accumulation of reserves in the taproots (expressed as percent dry weight) was already discussed and the data presented in Table 4 should be viewed within the context of persistence of seedlings established from seed. The high % dry weight of the primary seedling taproot is fairly consistent with regard to seed source but some intriguing trends emerge. The Liberty seedling roots appear to be stimulated at both NaCl and Na_2SO_4 salinities (excluding the 9 wk seedlings grown in a different growth chamber). At severe salinity stresses the % dry weight declined slightly but is statistically significant only in a few instances. The 'relative resistance' of the primary seedling taproot to salinity stresses was also evident in other seed source populations, grown under the standard conditions, as shown in Fig. 6 for 8-to-9 wk seedlings of the Dafoe and Watrous (1987) seed sources. Similarly,

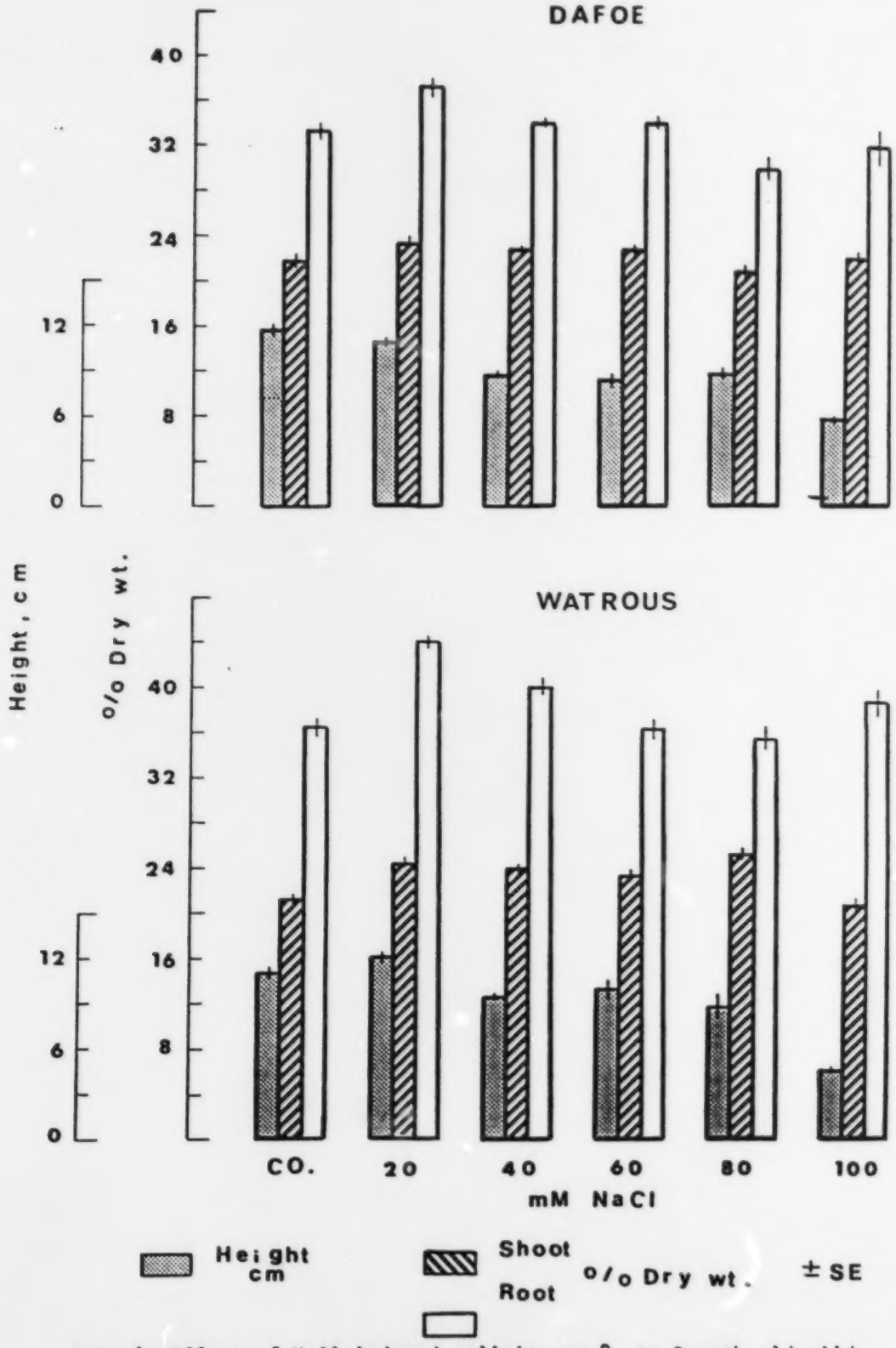

FIG. 6. Effect of NaCl-induced salinity on 8- to 9-week old wild licorice seedlings grown under the described standard conditions. Shown are stem height and percent dry weight of shoots and primary seedling taproots. Seed sources : Dafoe 1987 and Watrous 1987.

the percent dry weight of the shoot remained fairly constant up to 100 mM NaCl although stem height decreased from 40 mM onwards and was significantly depressed at the severe, 100 mM NaCl, salinity.

The data in Fig. 7 is of interest. The Bell seed source served as non-stressed control seeds. They were germinated on water, 4-day seedlings transplanted and raised under the standard growing conditions. Wild licorice (scarified) seeds become readily imbibed under high atmospheric humidity (Walther, 1982) as is likely to happen under natural conditions, even on salinized sites. Such 'water-raised' postgerminative seedlings would be less prone to early seedling root deterioration, promoting establishment. (Overall, the Bell seed source emerged as the most salt-tolerant among the seed sources tested). The data in Fig. 7 represents 1, 2, 3, and 8 wk seedling shoot growth. Survival of seedlings occurred up to 120 mM NaCl to 8wk. (Similarly, some Bell 1988 seedlings have survived for months in the growth chamber in 50 and 60 mM Na_2SO_4-enriched 1/2-strength nutrient solutions, and secondary shoots were periodically regenerated from adventitious buds). In the 8 wk seedlings stem height declined from 60 mM onwards but the decrease was more pronounced in the shoot mass (mg dry weight) exhibiting a near-linear decline from 20 mM NaCl onwards. But the percent dry weight (a reflection of the water status and dry matter accumulation in the shoot) was only pronounced at the severe salinities. The trend in the younger seedlings was similar but far less pronounced.

Mention should also be made of growing seedlings in vermiculite, irrigated with appropriate NaCl-enriched solutions, out-of-doors overwinter, and although the vermiculite was frozen solid, gradual (natural) thawing left some seedling taproots undamaged and secondary shoots developed from the overwintered adventitious buds in the spring of 1988. Amongst the surviving seedlings were those derived from Bell seed sources and raised in 60 mM NaCl, even a few in 80 mM but they remained small throughout the summer of 1989, while 20 mM NaCl and control seedlings showed profuse growth in spite of being pot-bound. Similarly, 9-to-10 wk seedlings raised in the growth chamber in nutrient solutions were

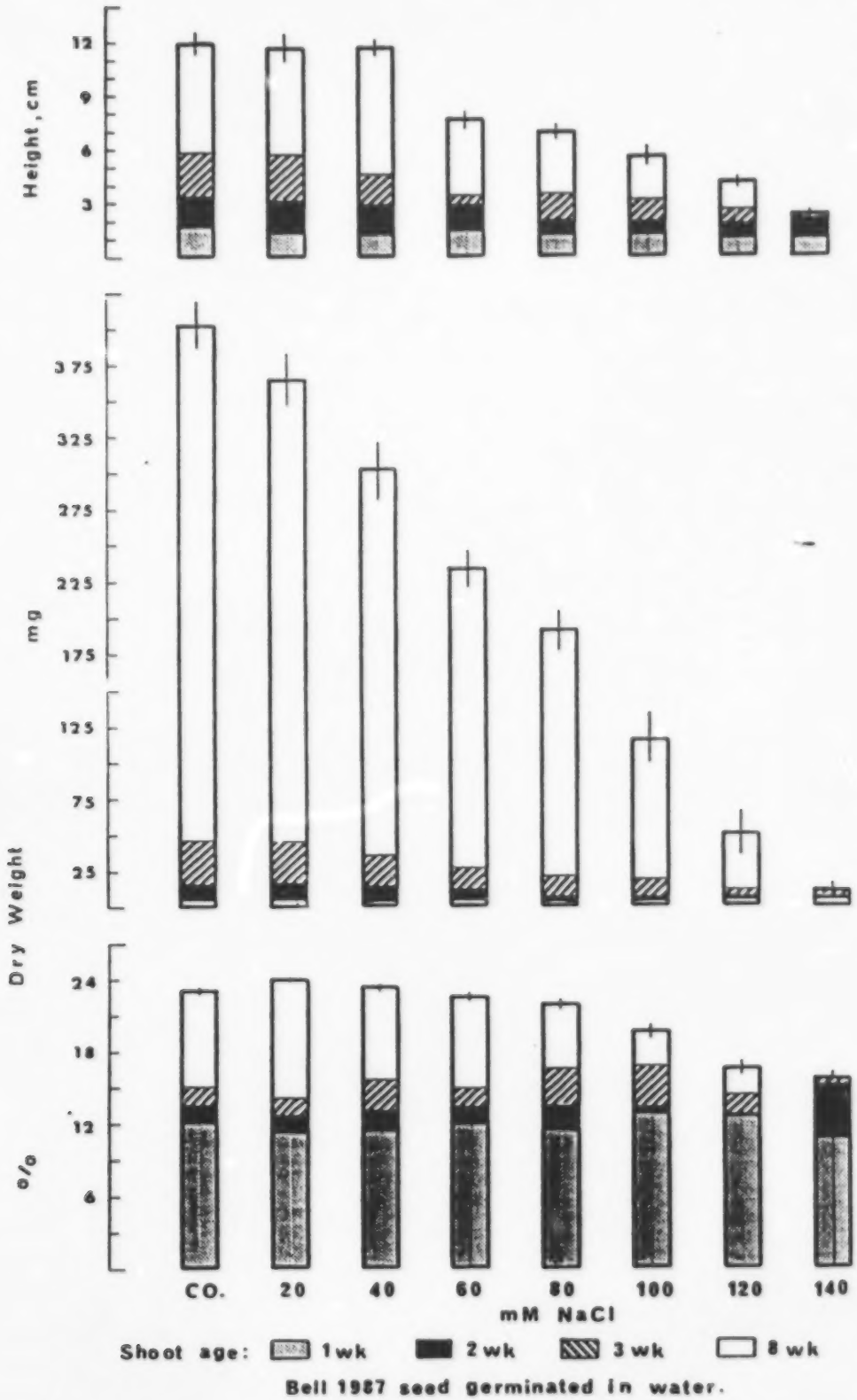

FIG. 7. Effect of NaCl-induced salinity on 1-, 2-, 3- and 8-week seedlings of wild licorice grown under the described standard conditions except seed were germinated in water. Shown are stem height, dry weight and percent dry weight of shoots.

transplanted into soil (Biology Garden) early enough in the fall of 1987 and 1988 to allow for establishment of the root systems. The seedlings included controls and up to 60 mM NaCl-stressed ones. The 1987 transplants did survive overwinter; the controls and lower salinity-stressed seedlings grew well next spring, although some retardation was apparent in the NaCl-stressed plants. After surviving a second winter, the 1987 transplants, flowered and set seed in 1989 in a manner comparable to field plants in those derived from control and 20 mM NaCl seedlings, less flowering occurred in the other initially NaCl-stressed plants. The 1988 transplants have not flowered in the 1989 season, confirming the trend observed in the Bell population in prior years. Isolated experiments were also carried out with seed planted in soil (control soil mentioned before) and seedlings raised in growth chambers. They were periodically given 20 to 100 mM NaCl-enriched nutrient solution to simulate 'salt shock'. Retardation in growth was generally noticeable after about 10 - 12 days, and when the plants were watered excessively to 'wash out the salt' (as is likely after a heavy rain), corresponding recovery was similar except in the seedlings stressed with 60, 80 or 100 mM NaCl which did not attain growth equivalent to those stressed with only 20 or 40 mM. Limited growth chamber space accounted for the discontinuation of these experiments but a few plants were kept and after about 2 years no sign of flowering has appeared, paralleling failing attempts to raise wild licorice plants under indoor conditions to the flowering stage (lack of natural photoperiodic changes and/or a prolonged cold period are probable reasons for this failure to flower).

CONCLUSIONS AND IMPACT

The data on the germination behaviour of various seed batches of wild licorice under artificially induced sodium chloride or sodium sulfate stress is separable into two phases: high initial germinability at all salt concentrations and a decline to near or less than 50% in the development of viable immediate postgerminative seedlings under severe salinity stress. This

indicates that under field conditions, high germination will not translate into equivalent seedling establishment success on severely or very severely salinized sites. The growth and development of transplanted viable seedlings under the described controlled standard conditions was also adversely affected at severe salinities but no morphological deviations, relative to the controls and low-salinity seedlings, were observed, only retardation of growth. This was manifested in the measured parameters: seedling leaf chlorophyll, stem height and shoot mass. Enlargement of the primary seedling root due to deposition of reserves into a taproot was least affected, although formation of adventitious buds on 5-week or older seedlings was less intense under severe salinity stress. It indicates, however, that survival under saline conditions to this developmental stage (taproot enlargement and production of adventitious buds giving rise to secondary shoots) will ensure establishment on severely stressed sites.

Germination trials of selected seed sources in soil both in-doors and out-of-doors reflected the behaviour under the described standard conditions. In soil mixtures with conductivities approaching 14 mS cm^{-1} the depressing effect on germination and seedling growth was in part due to the loss of structure in the highly salinized stock soil. Retention of soil structure would serve as an ameliorating effect on the salinity stress under field conditions.

The response to the artificially induced salinity stress and to that of the saline soil mixtures was not uniform amongst the nineteen seed batches tested (comprising fourteen different sites, five of which were represented by two crop years). The differential response by seed sources is here ascribed to the prevailing site conditions during seed formation and the inherent variability within natural wild licorice populations. In conclusion, it is emphasized here that in general the sulfate ion exerted a more depressing effect than either the chloride or sodium ions, notwithstanding the fact that most seed batches originated on soils dominated by sulfate salts.

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